REMARKS

Applicants respectfully request reconsideration of the present application.

CLAIMS STATUS

Pending claims include rejected claims 32-33 and 35 and withdrawn claims 1-31, 34 and 36-37.

REJECTION UNDER 35 U.S.C. § 103(a)

Claims 32-33 and 35 stand rejected over of Jacob (WO99/24401) in view of Hollingsworth *et. al.* (US 6,462,197) and further in view of van den Broek *et. al.* (Recl. Trav. Chim. Pay-bas, 1994, pp. 507-516). Applicants respectfully traverse.

Applicants note that Hollingsworth is not prior art against the claimed invention. The PTO cites Hollingsworth as disclosing 1,5-imino-1,5,6-trideoxy alditols with D-galacto configuration including 1,5-imino-1,5,6-trideoxy alditol substituted with alkyl moiety, see Office Action, page 4. The earliest possible priority date of Hollingsworth is March 31, 2000, the filing date of provisional application No. 60/193,554.

The present application claims priority to provisional application No. 60/148,101 filed August 10, 1999, prior to the earliest possible priority date of Hollingsworth. Applicants enclose with this communication a copy of the '101 application. Applicants note that the '101 application contains all the disclosure in Hollingsworth, on which the PTO relies in rejecting the present claims. Applicants refer to "Summary of the Invention" section on pages 3-6 of the '101 application and, more specifically, to page 6, lines 10-11, which recite N-nonyl-1,5,6-trideoxy-1,5-imino-D-galactitol (N-nonyl MeDGJ). Applicants also note that Example 1 on pages 16-17 of the 101 application details the synthesis of N-nonyl MeDGJ.

In the absence of Hollingsworth, the two remaining prior art references against the pending claims are Jacob and van den Broek. As the PTO admitted, Jacob fails to teach either N-nonyl-1,5,6-trideoxy-1,5 imino-D-galactitol or N-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol compounds, see Office Action, page 3. Furthermore, Jacob does not disclose any **N-substituted 1,5,6-trideoxy-1,5-imino-D-galactitol compounds**, including **N-alkylated 1-methyl-deoxygalactonojirimycin (MeDGJ) compounds** recited in rejected claim 32. Van den Broek does not teach any N-substituted 1,5,6-trideoxy-1,5-imino-D-

galactitol compounds either. Although Jacob discloses N-substituted 1,5-dideoxy-1,5-imino-D galactitol compounds, neither Jacob, nor van der Broek provide any required motivation to modify Jacob's compounds into **N-substituted 1,5,6-trideoxy-1,5-imino-D-galactitol compounds** or **N-alkylated 1-methyl-deoxygalactonojirimycin (MeDGJ) compounds** recited in rejected claim 32. Along the same lines, although van der Broek teaches N-(7-oxadecyl)-1-deoxynojirimycin and N-decyl-1-deoxynojirimycin compounds, neither Jacob, nor van der Broek provide any required motivation to modify van der Broek's compounds into **N-substituted 1,5,6-trideoxy-1,5-imino-D-galactitol compounds** or **N-alkylated 1-methyl-deoxygalactonojirimycin (MeDGJ) compounds** recited in rejected independent claim 32. In conclusion, Applicants request withdrawal of the rejection.

CONCLUSION

Applicants believe that the present application is in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. § 1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date October 13, 2006

By _____ Reg. #56,439
Stephen B. Maebius Alexey Sapriji
Attorney for Applicant
Registration No. 35,264

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING PROVISIONAL PATENT APPLICATION

Under 35 USC 111(b)
(Not for DESIGN cases)

Box:
PROVISIONAL
APPLICATION

Asst. Commissioner of Patents
Washington, D.C. 20231

PROVISIONAL APPLICATION
Under Rule 53(c)

Sir:

(Our Deposit Account No. 03-3975)

Herewith is a PROVISIONAL APPLICATION
Title: LONG CHAIN N-ALKYL . . . THEREOF

Our Order No. 81361 (C#) | 243447 (M#)

Atty. Dkt. PMS 243447 (M#) | Client Ref

Date: 8/10/99

including:

1. Specification: 20 pages
2. ☐ Specification in non-English language
3. ☒ Drawings: 7 sheet(s)

4. The invention ☐ was ☒ was not made by, or under a contract with, an agency of the U.S. Government.
If yes, Government agency/contact # = _____
5. ☐ Attached is an assignment and cover sheet. Please return the recorded assignment to the undersigned.
6. ☐ Attached: _____ (No.) Verified Statement(s) establishing "small entity" status under Rules 9 & 27.
NOTE: Do NOT File IDS!
7. ☒ Attached: Claims 1-47; Abstract

8. This application is made by the following named inventor(s) **(Double check instructions for accuracy.):**

	First	Middle Initial	Family Name
(1) Inventor	Nicole		ZITZMAN
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(5) Inventor	George	W.J.	FLEET
Residence	Oxfordshire (City)	United Kingdom (State/Foreign Country)	

9. **NOTE: FOR ADDITIONAL INVENTORS, check box ☒ and attach sheet (PAT102A) with same information regarding additional inventors.**

REQUEST FOR FILING APPLICATION
Under 35USC 111(b)
(Continued : Additional Inventors)

(6) Inventor	Raymond	A.	DWEK
	First	Middle Initial	Family Name
Residence	Oxfordshire	United Kingdom	
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(8) Inventor			
	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	

(9) Inventor			
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Residence			
	City	State/Foreign Country	

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	City	State/Foreign Country	

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	City	State/Foreign Country	

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Residence			
	City	State/Foreign Country	

(13) Inventor			
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Residence			
	City	State/Foreign Country	

(14) Inventor			
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Residence			
	City	State/Foreign Country	

	Large/Small Entity		Fee Code
10. Filing Fee	$150/$75	+150	114/214
11. If "non-English" box 2 is X'd, add Rule 17(k) processing fee	$130	+0	139
12. If "assignment" box 5 is X'd, add recording fee.	$40	+0	581
13. TOTAL FEE ENCLOSED =		$150	

<u>CHARGE STATEMENT</u>: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-17 (<u>missing or insufficient fee only</u>) now or hereafter relative to this application or credit any overpayment, to our Account/Order Nos. shown in the heading hereof for which purpose a <u>duplicate</u> copy of this sheet is attached.

Pillsbury Madison & Sutro LLP
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NOTE: File in <u>duplicate</u> with 2 post card receipts (PAT-103) & attachments

APPLICATION UNDER UNITED STATES PATENT LAWS

Invention: LONG CHAIN N-ALKYL COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS THEREOF

Inventor(s): **Nicole ZITZMAN, Terry D. BUTTERS, Frances PLATT, Sandra CARROUEE, George W.J. FLEET, Raymond A. DWEK**

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Telephone: (202) 861-3000

This is a:

- ☒ Provisional Application
- ☐ Regular Utility Application
- ☐ Continuing Application
- ☐ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification
  Sub. Spec. filed _____
  in App. No _____/_____
- ☐ Marked Up Specification re
  Sub. Spec. filed _____
  in App. No. _____/_____

SPECIFICATION

LONG CHAIN N-ALKYL COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS THEREOF

FIELD OF THE INVENTION

The invention relates to long chain N-alkyl amino and imino compounds and pharmaceutical compositions thereof.

BACKGROUND OF THE INVENTION

More than 40 million people worldwide are chronically infected with the hepatitis C virus (HCV), and this represents one of the most serious threats to the public health of developed nations (Hoofnagle *et al.* (1997) New Engl. J. Med. 336:347-356). Hepatitis C infection is the cause of more than 10,000 deaths annually in the United States (*Hepatitis C Treatment*, Washington Post, November 11, 1997, at A2), a number that is expected to triple in the next twenty years in the absence of effective intervention. Chronic HCV also increases the risk of liver cancer. There are more than 40 million people worldwide who are chronically infected with HCV, representing one of the most serious threats to the public health of developed nations (Hoofnagle *et al.* (1997) New Engl. J. Med. 336:347-356). Persistent infection develops in as many as 85% of HCV patients and in at least 20% of these patients the chronic infection leads to cirrhosis within twenty years of onset of infection. With an estimated 3.9 million North Americans chronically infected, complications from hepatitis C infection are now the leading reasons for liver transplantation in the United States.

Therapeutic interventions which are effective for treatment of HCV infection are limited in number and effectiveness. Standard treatment for HCV infection includes administration of interferon-alpha. However, interferon-alpha is of limited use in about 20% of the HCV-infected population (Hoofnagle *et al.* (1997) New Engl. J. Med. 336:347-356) and treatment with this compound results in long-term improvement in only 5% of patients. Furthermore, the complications and limitations of interferon-alpha seriously limit the applicability of the treatment. An experimental treatment comprising administration of interferon-alpha and ribavirin (1-β-*D*-ribofuranosy1-1H-1,2,4-triazole-3-carboxamide) resulted in long-term improvement in only half of the patients suffering a relapse of HCV infection (*Hepatitis C Treatment*, Washington Post, November 11, 1997, at A2). Clearly, the disappointing results with interferon must prompt a search for more effective and less toxic

therapeutics. Thus, a critical need remains for a therapeutic intervention that effectively treats HCV infection or supplements those otherwise available.

In addition to those people chronically infected with HCV, there are more than 350 million people chronically infected with Hepatitis B virus (HBV). More than 150 million of these people are likely to die from liver disease in the absence of intervention. As many as 20 million HBV carriers reside in developed nations, as do most HCV carriers.

A large number of individuals who are infected with HCV are also infected with HBV. The therapy for combined HBV/HCV infection is particularly challenging because the HBV and HCV viruses differ from one another in therapeutically significant ways. HBV is a hepadnavirus, while HCV is a pestivirus. HBV is a DNA-containing virus, the genome of which is replicated in the nucleus of the infected cell using a combination of a DNA-dependent RNA polymerase and an RNA-dependent DNA polymerase (i.e., a reverse transcriptase). HCV is an RNA-containing virus, the genome of which is replicated in the cytoplasm of the infected cell using one or more types of RNA-dependent RNA polymerases. Despite the frequent concurrence of HBV infection and HCV infection, a number of compounds known to be effective for treating HBV infection are not effective against HCV. For example, lamivudine (the nucleoside analog 3TC) is useful for treating HBV infection, but is not useful for treating HCV infection. The difference in the susceptibility of HBV and HCV to antiviral agents no doubt relates to their genetically based replicative differences. There remains a particularly critical need for a therapeutic intervention that effectively treats both HBV and HCV infection.

Animal viruses can cause significant losses to the livestock industry (Sullivan *et al.* (1995) Virus Res. 38:231-239). Such animal viruses include pestiviruses and flaviviruses such as bovine viral diarrhea virus (BVDV), classical swine fever virus, border disease virus, and hog cholera virus.

HCV is an RNA virus belonging to the *Flaviviridae* family. Individual isolates consist of closely related, yet heterologous populations of viral genomes. This genetic diversity enables the virus to escape the host's immune system, leading to a high rate of chronic infection. The flavivirus group to which HCV belongs is known to include the causative agents of numerous human diseases transmitted by arthropod vectors. Human diseases caused by flaviviruses include various

hemorrhagic fevers, hepatitis, and encephalitis. Viruses known to cause these diseases in humans have been identified and include, for example, yellow fever virus, dengue viruses 1-4, Japanese encephalitis virus, Murray Valley encephalitis virus, Rocio virus, West Nile fever virus, St. Louis encephalitis virus, tick-borne encephalitis virus, Louping ill virus, Powassan virus, Omsk hemorrhagic fever virus, and Kyasanur forest disease virus. A critical need therefore also exists for treating animals, as well as humans, infected with at least one virus, such as a flavivirus and/or pestivirus.

SUMMARY OF THE INVENTION

In general, the invention features long chain N-alkyl amino and imino compounds and pharmaceutical compositions including long chain N-alkyl amino and imino compounds. The long chain N-alkyl group is a C_8-C_{16} alkyl group. The long chain N-alkyl compounds can be used in the treatment of viral infections in a cell or an individual. In an individual, the infection may result in chronic or acute disease. The long chain N-alkyl amino compounds may or may not inhibit glycosidase activity or glycoplipid synthesis at a detectable level. For example, the long chain N-alkyl compounds can be derived from a piperidine, a pyrrolidine, a phenylamine, a pyridine, a pyrrole, or an amino acid.

In one aspect, the invention features a nitrogen-containing virus-inhibiting compound including an N-C_8-C_{16} alkyl group. Preferably, the compound includes an N-C_8-C_{10} alkyl group, such as a nonyl group. The nitrogen-containing virus-inhibiting compound can have an IC_{50} of about 20 μM or less, preferably about 10 μM or less, for the inhibition of BVDV.

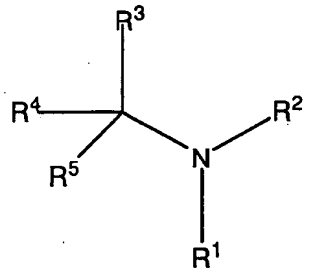
In another aspect, the invention features a method of inhibiting morphogenesis of a virus. The method includes administering an effective amount of the nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt thereof, to a cell or an individual infected with the virus. The cell can be a mammalian host cell or a human host cell.

In yet another aspect, the invention features a method of treating an individual infected with a virus. The method includes administering an effective amount of the nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt

thereof, to an individual infected with a virus. The treatment can reduce, abate, or diminish the virus infection in the animal. The animal can be a mammal, such as a pig, a cow or, particularly, a human being. The individual can be a mammal, such as a human. The nitrogen-containing virus-inhibiting compound can be administered orally.

In another aspect, the invention features a method of manufacturing a pharmaceutical composition comprising combining at least one nitrogen-containing virus-inhibiting compound including an N-C_8-C_{16} alkyl group with a pharmaceutically acceptable carrier.

The compound can have the formula:



in which R^1 is a C_8-C_{16} alkyl;

R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, or R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, an aroyloxy, or deleted;

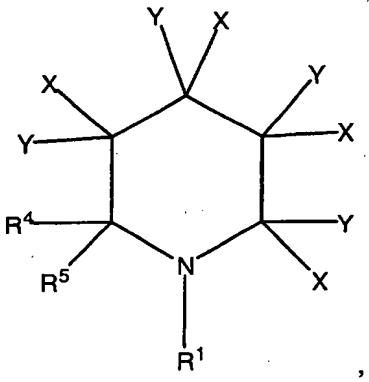
R^4 is hydrogen or deleted; and

R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy, or R^3 and R^5, together, form a phenyl and R^4 is deleted. When R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted, all Y are deleted. The compound can be a physiologically acceptable salt or solvate of the compound.

In certain embodiments, R^1 is a C_8-C_{10} alkyl (e.g., C_9 alkyl) and R^2 can be hydrogen, R^3 can be carboxy, or a C_1-C_4 alkoxycarbonyl, R^4 can be hydrogen, and R^5 can be hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl,

an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy. In certain preferred embodiments, R^3 is carboxy. In other preferred embodiments, R^3 and R^5, together, form a phenyl and R^4 is deleted. In yet other preferred embodiments, R^2 and R^3, together, are $-(CXY)_n-$.

In certain embodiments, the compound has the formula:



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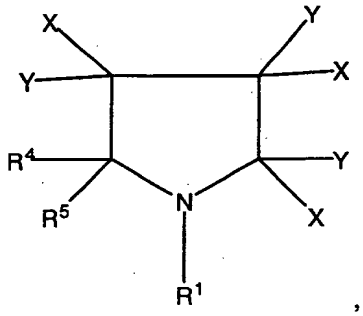

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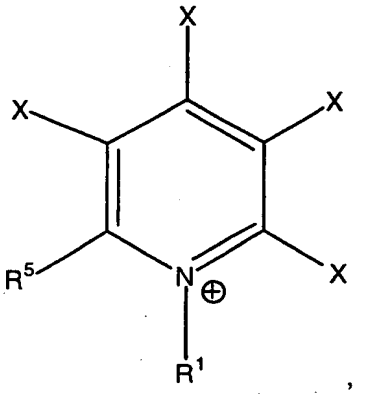

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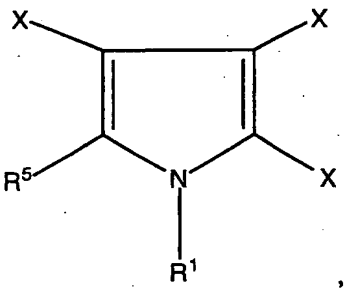

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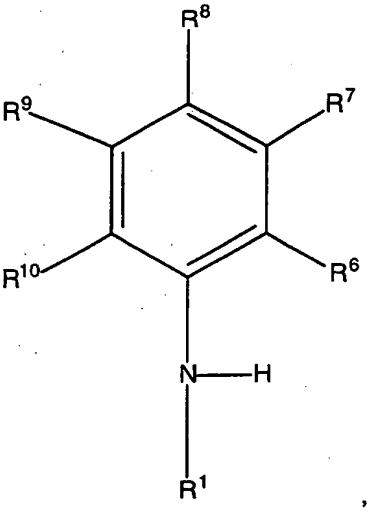
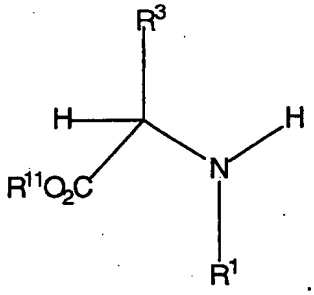


, or



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Each of R^6-R^{10}, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and R^{11} is hydrogen, or a C_1-C_4 alkyl.

The nitrogen-containing virus inhibiting compound can be an N-alkylated piperidine, an N-alkylated pyrrolidine, an N-alkylated phenylamine, an N-alkylated pyridine, an N-alkylated pyrrole, or an N-alkylated amino acid. In certain embodiments, the N-alkylated piperidine or the N-alkylated pyrrolidine can be an imino sugar. In preferred embodiments, the nitrogen-containing virus-inhibiting compound can be N-nonyl-deoxygalactonojirimycin (N-nonyl-1,5-dideoxy-1,5-imino-D-galactitol or N-nonyl DGJ), N-nonyl-1,5,6-trideoxy-1,5-imino-D-galactitol (N-nonyl MeDGJ), N-nonyl altrostatin, N-nonyl-*2R,5R*-dihydroxymethyl-*3R,4R*-dihydroxypyrrolidine (N-nonyl DMDP), N-nonyl-deoxynojirimycin (N-nonyl DNJ), or N-nonyl-2-aminobenzamide (2ABC9), or a derivative, an enantiomer or a stereoisomer thereof. The structures of these compounds are shown in Figure 1.

In certain embodiments, the virus can be a flavivirus or a pestivirus. Infections by flaviviruses include, but are not limited to, those caused by a yellow fever virus, a dengue virus (e.g., dengue viruses 1-4), a Japanese encephalitis virus, a Murray Valley encephalitis virus, a Rocio virus, a West Nile fever virus, a St. Louis encephalitis virus, a tick-borne encephalitis virus, a Louping ill virus, a Powassan virus, an Omsk hemorrhagic fever virus, and a Kyasanur forest disease virus. Infections by pestiviruses include, but are not limited to, those caused by Hepatitis C virus (HCV), rubella virus, a bovine viral diarrhea virus (BVDV), a classical swine fever virus, a border disease virus, or a hog cholera virus.

According to yet another aspect, the invention features a prophylactic method for protecting a mammal infected by a virus from developing hepatitis or a heptacellular cancer that is among the sequelae of infection by the virus, including administering to the virus infected cell of the animal an effective anti-viral amount of the nitrogen-containing virus-inhibiting compound.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph depicting chemical structures for compounds which are inhibitory.

Figure 2 is a graph depicting the percent of BVDV plaques produced by an infected cell culture in the presence of various concentrations of N-nonyl compounds.

Figure 3 is a graph depicting the IC_{50} of various alkyl lengths of N-alkylated compounds in the inhibition of BVDV.

Figure 4 is a graph depicting the percent of BVDV plaques produced by an infected cell culture in the presence of N-nonyl DGJ or N-decyl DGJ.

Figure 5 is a graph depicting the IC_{50} of N-nonyl compounds for inhibition of BVDV.

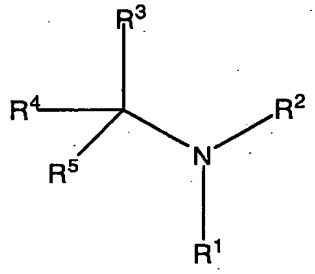
Figure 6 is a graph depicting the percent of BVDV plaques produced by an infected cell culture in the presence of N-nonyl amino compounds.

Figure 7 is a graph depicting the comparative cellular uptake of radioactively labeled inhibitors.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The nitrogen-containing virus-inhibiting compound includes an N-C_8-C_{16} alkyl group, such as an N-C_8-C_{10} alkyl group, particularly a nonyl group. The nitrogen-containing virus-inhibiting compound can be an N-alkylated piperidine, an N-alkylated pyrrolidine, an N-alkylated phenylamine, an N-alkylated pyridine, an N-alkylated pyrrole, or an N-alkylated amino acid, such as N-nonyl DGJ, N-nonyl MeDGJ, N-nonyl altrostatin, N-nonyl DMDP, N-nonyl DNJ, or N-nonyl-2-aminobenzamide.

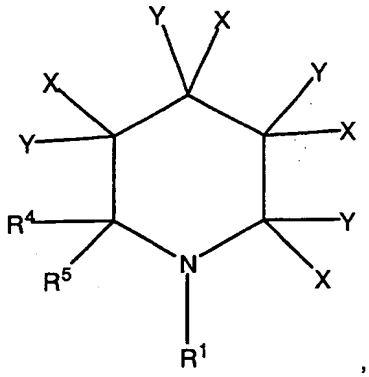
The compound can have the formula:



in which R^1 is a C_8-C_{16} alkyl, R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, R^4 is hydrogen, and R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy. Alternatively, R^1 is a C_8-C_{16}

alkyl, R^2 is hydrogen, R^3 and R^5, together, form a phenyl, which can be substituted or unsubstituted, and R^4 is deleted. In another alternative, R^1 is a C_8-C_{16} alkyl, R^4 is hydrogen or deleted, R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy, and R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, an aroyloxy, or deleted. When R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted, all Y are deleted. The compound can be a physiologically acceptable salt or solvate of the compound.

In certain embodiments, the compound has the formula:



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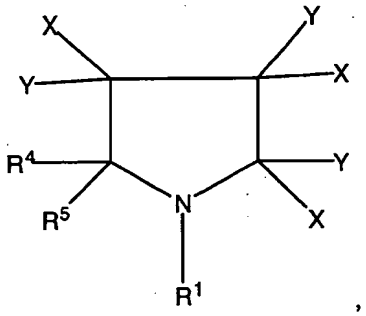

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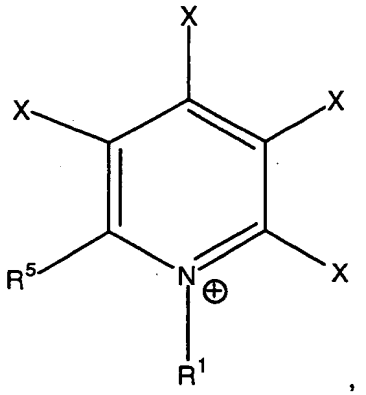

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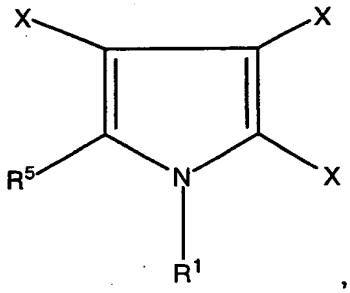

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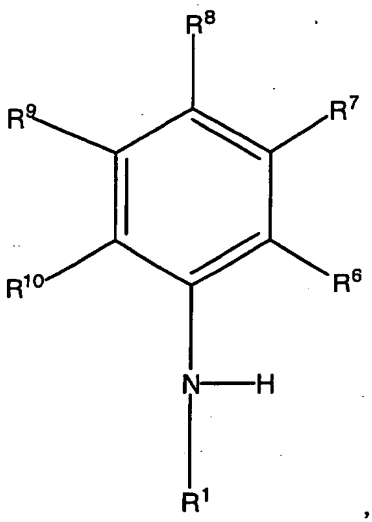

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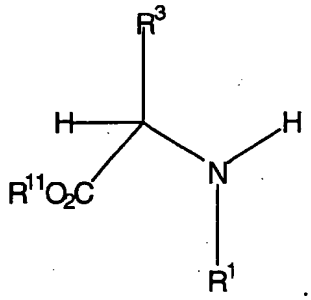


Each of R^6-R^{10}, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and R^{11} is hydrogen, or a C_1-C_4 alkyl.

As used herein, the groups have the following characteristics, unless the number of carbon atoms is specified otherwise. Alkyl groups have from 1 to 16 carbon atoms and are linear or branched, substituted or unsubstituted. Alkoxy groups have from 1 to 16 carbon atoms, and are linear or branched, substituted or unsubstituted. Alkoxycarbonyl groups are ester groups having from 2 to 16 carbon atoms. Alkenyloxy groups have from 2 to 16 carbon atoms, from 1 to 6 double bonds, and are linear or branched, substituted or unsubstituted. Alkynyloxy groups have from 2 to 16 carbon atoms, from 1 to 3 triple bonds, and are linear or branched, substituted or unsubstituted. Aryl groups have from 6 to 14 carbon atoms (e.g., phenyl groups) and are substituted or unsubstituted. Aralkyloxy (e.g., benzyloxy) and aroyloxy (e.g., benzoyloxy) groups have from 7 to 15 carbon atoms and are substituted or unsubstituted. Amino groups can be primary, secondary, tertiary, or quaternary amino groups (i.e., substituted amino groups). Aminocarbonyl groups are amido groups (e.g., substituted amido groups) having from 1 to 32 carbon atoms. Substituted groups can include a substituent selected from the group consisting of halogen, hydroxy, $C_{1\text{-}10}$ alkyl, $C_{2\text{-}10}$ alkenyl, $C_{1\text{-}10}$ acyl, or $C_{1\text{-}10}$ alkoxy.

The N-alkylated amino acid can be an N-alkylated naturally occurring amino acid, such as an N-alkylated α-amino acid. A naturally occurring amino acid is one of

the 20 common α-amino acids (Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Asn, Lys, Glu, Gln, Arg, His, Phe, Cys, Trp, Tyr, Met, and Pro), and other amino acids that are natural products, such as norleucine, ethylglycine, ornithine, methylbutenyl-methylthreonine, and phenylglycine. Examples of amino acid side chains (e.g., R^5) include H (glycine), methyl (alanine), $-CH_2C(O)NH_2$ (asparagine), $-CH_2$-SH (cysteine), and $-CH(OH)CH_3$ (threonine).

The long chain N-alkylated nitrogen-containing compound can be prepared by reductive alkylation of an amino (or imino) compound. For example, the amino or imino compound can be exposed to a long chain aldehyde, along with a reducing agent (e.g., sodium cyanoborohydride) to N-alkylate the amine.

The compounds can include protecting groups. Various protecting groups are well known. In general, the species of protecting group is not critical, provided that it is stable to the conditions of any subsequent reaction(s) on other positions of the compound and can be removed at the appropriate point without adversely affecting the remainder of the molecule. In addition, a protecting group may be substituted for another after substantive synthetic transformations are complete. Clearly, where a compound differs from a compound disclosed herein only in that one or more protecting groups of the disclosed compound has been substituted with a different protecting group, that compound is within the invention. Further examples and conditions are found in T. W. Greene, *Protective Groups in Organic Chemistry*, (1st ed., 1981, Theodora Greene and P. G. Wuts, 2nd ed., 1991).

The compounds can be purified, for example, by crystallization or chromatographic methods. The compound can be prepared stereospecifically using a stereospecific amino or imino compound as a starting material.

The amino and imino compounds used as starting materials in the preparation of the long chain N-alkylated compounds are commercially available (Sigma, St. Louis, MO, Cambridge Research Biochemicals, Norwich, Cheshire, U.K. or Toronto Research Chemicals, Ontario, Canada) or can be prepared by known synthetic methods. For example, the compounds can be long chain N-alkylated imino sugar compounds. The imino sugar can be, for example, deoxygalactonojirmycin (DGJ), 1-methyl-deoxygalactonojirimycin (MeDGJ), deoxynorjirimycin (DNJ), altrostatin, 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP), or derivatives, enantiomers, or stereoisomers thereof.

The synthesis of a variety of imino sugar compounds have been described. For example, methods of synthesizing DNJ derivatives are known and are described, for example, in U.S. Patent Nos. 5,622,972, 5,200,523, 5,043,273, 4,994,572, 4,246,345, 4,266,025, 4,405,714, and 4,806,650, and U.S. patent application 07/851,818, filed March 16, 1992. Methods of synthesizing other imino sugar derivatives are known and are described, for example, in U.S. Patent Nos. 4,861,892, 4,894,388, 4,910,310, 4,996,329, 5,011,929, 5,013,842, 5,017,704, 5,580,884, 5,286,877, and 5,100,797. The enantiospecific synthesis of 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) is described by Fleet and Smith, Tetrahedron Lett. 26(11), 1469-1472 (1985).

The substituents on the imino sugar compound can influence the potency of the compound as an antiviral agent and additionally can preferentially target the molecule to one organ rather than another. Methods for comparing the potencies of various substituted compounds are provided in the Examples.

With the exception of the pyridinium compounds, which are in salt form, the compounds described herein may be used in the free amine form or in a pharmaceutically acceptable salt form. The counter anion of the pyridinium compound can be chloride, tartrate, phosphate, or sulfate. Pharmaceutical salts and methods for preparing salt forms are provided in Berge, S. *et al.* (1977) J. Pharm. Sci. 66(1):1-18. Pharmaceutically acceptable salts can be preferred for compounds that are difficult to solubilize in the pharmaceutical composition (e.g., compounds having longer alkyl chains). A salt form is illustrated, for example, by the HCl salt of an amino derivative. The compounds may also be used in the form of prodrugs, such as the 6-phosphorylated DNJ derivatives described in U.S. Patents Nos. 5,043,273 and 5,103,008. Use of compositions which further comprise a pharmaceutically acceptable carrier and compositions which further comprise components useful for delivering the composition to an animal are explicitly contemplated. Numerous pharmaceutically acceptable carriers useful for delivering the compositions to a human and components useful for delivering the composition to other animals such as cattle are known in the art. Addition of such carriers and components to the composition of the invention is well within the level of ordinary skill in the art. For example, the compounds can be di- or tetra- acetates, propionates, butyrates, or isobutyrates. The compound can be a solvate.

The invention also encompasses isotopically-labeled counterparts of compounds disclosed herein. An isotopically-labeled compound of the invention has one or more atoms replaced with an isotope having a detectable particle- or x-ray-emitting (radioactive) nucleus or a magnetogyric nucleus. Examples of such nuclei include ^{2}H, ^{3}H, ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P, ^{32}P and ^{125}I. Isotopically-labeled compounds of the invention are particularly useful as probes or research tools for spectrometric analyses, radioimmunoassays, binding assays based on scintillation, fluorography, autoradiography, and kinetic studies such as inhibition studies or determination of primary and secondary isotope effects.

The nitrogen-containing virus-inhibiting compound can be administered to a cell or an individual affected by a virus. The compound can inhibit morphogenesis of the virus, or it can treat the individual. The treatment can reduce, abate, or diminish the virus infection in the animal. For example, the N-nonyl compounds are antiviral. The antiviral activity is substantially unrelated to the remaining functionalities of the compound.

The nitrogen-containing virus-inhibiting compound combined with at least one other antiviral compound, such as an inhibitor of a viral DNA or RNA polymerase and/or protease, and/or at least one inhibitor of expression of viral genes, replication of the viral genome, and/or assembly of a viral particle. The supplemental antiviral compound may be any antiviral agent, which is presently recognized, or any antiviral agent which becomes recognized. By way of example, the supplemental antiviral compound may be interferon-alpha, interferon-beta, ribavirin, lamivudine, brefeldin A, monensin, TUVIRUMAB™ (Protein Design Labs) PENCICLOVIR™ (SmithKline Beecham), FAMCICLOVIR™ (SmithKline Beecham), BETASERON™ (Chiron), THERADIGM-HBV™ (Cytel), Adefovir Dipivoxil (GS 840, Gilead Sciences), INTRON A™ (Schering Plough), ROFERON™ (Roche Labs), BMS 200,475 (Bristol Myers Squibb), LOBUCAVIR™ (Bristol Myers Squibb), FTC (Triangle Pharmaceuticals), DAPD (Triangle Pharmaceuticals), thymosin alpha peptide, Glycovir (Block *et al.* (1994) Proc. Natl. Acad. Sci. 91:2235-2240), granulocyte macrophage colony stimulating factor (Martin *et al.* (1993) Hepatology 18:775-780), an "immune-cytokine" (Guidotti *et al.* (1994) J. Virol. 68:1265-1270), CDG (Fourel *et al.* (1994) J. Virol. 68:1059-1065), or the like.

Long chain N-alkyl compounds are agents that exhibit an inhibitory effect on

viral expression. While certain short chain N-alkyl derivatives of imino sugars (e.g., N-butyl DNJ) are potent inhibitors of the N-linked oligosaccharide processing enzymes, such as α-glucosidase I and α-glucosidase II (Saunier *et al.* (1982) J. Biol. Chem. 257:14155-14161; Elbein (1987) Ann. Rev. Biochem. 56:497-534). Some long chain N-alkyl compounds of the invention may exhibit substantially little or no inhibition of a glycosidase enzyme, especially in comparison with N-butyl DNJ. Unexpectedly, some long chain N-alkyl compounds do effectively inhibit viral morphogenesis in cells infected with a virus, such as a flavivirus or pestivirus. For example, the N-nonyl nitrogen-containing virus-inhibiting compound can have an IC_{50} of about 10 μM or less, preferably about 3 μM or less, for the inhibition of BVDV or another virus, but the same compounds may exhibit little activity against glycosidases or inhibition of glycolipid synthesis.

Methods for treating a mammal infected with respiratory syncytial virus (RSV) using DNJ derivatives have been described in U.S. Patent No. 5,622,972. The use of DNJ and N-methyl-DNJ has also been disclosed to interrupt the replication of non-defective retroviruses such as human immunodeficiency virus (HIV), feline leukemia virus, equine infectious anemia virus, and lentiviruses of sheep and goats (U.S. Patents Nos. 5,643,888 and 5,264,356; Acosta *et al.* (1994) Am. J. Hosp. Pharm. 51:2251-2267).

In the absence of a suitable cell culture system able to support replication of human HCV, bovine viral diarrhea virus (BVDV) serves as the FDA approved model organism for HCV, as both share a significant degree of local protein region homology (Miller *et al.*, (1990) Proc. Natl. Acad. Sci. 87:20571), common replication strategies, and probably the same sub-cellular location for viral envelopment. Compounds found to have an antiviral effect against BVDV are highly recommended as potential candidates for treatment of HCV.

The cytotoxicity resulting from exposure of mammalian cells in tissue culture to bovine viral diarrhea virus (BVDV) is prevented by addition of a nitrogen-containing virus-inhibiting compound to the tissue culture medium. The virus inhibitors that were used in the examples below included long chain N-alkyl derivatives of DGJ. For example, N-nonyl DGJ (NN-DGJ) has an IC_{50} of about 7 μM and N-decyl DGJ has an IC_{50} of about 2.5 μM against BVDV. Because BVDV is an accepted tissue culture model of HCV (Henzler and Kaiser (1998) Nature Biotech.

16:1077-1078), the compositions and methods described herein for inhibiting morphogenesis of BVDV are also useful for inhibiting morphogenesis of HCV.

The amount of antiviral agent administered to an animal or to an animal cell according to the methods of the invention is an amount effective to inhibit the viral morphogenesis from the cell. The term "inhibit" as used herein refers to the detectable reduction and/or elimination of a biological activity exhibited in the absence of a nitrogen-containing virus-inhibiting compound according to the invention. The term "effective amount" refers to that amount of composition necessary to achieve the indicated effect. The term "treatment" as used herein refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder in a subject who is free therefrom.

Thus, for example, treatment of viral infection includes destruction of the infecting agent, inhibition of or interference with its growth or maturation, neutralization of its pathological effects, and the like. The amount of the composition which is administered to the cell or animal is preferably an amount that does not induce any toxic effects which outweigh the advantages which accompany its administration.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient.

The selected dose level will depend on the activity of the selected compound, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound(s) at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to four doses per day. It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors, including the body weight, general health, diet, time and route of administration and combination with other drugs and the severity of the disease being treated. It is expected that the adult human daily dosage will normally range from between about one microgram to about one gram, preferably

from between about 10 mg and 100 mg, of the nitrogen-containing virus-inhibiting compound per kilogram body weight. Of course, the amount of the composition which should be administered to a cell or animal is dependent upon numerous factors well understood by one of skill in the art, such as the molecular weight of the nitrogen-containing virus-inhibiting compound, the route of administration, and the like.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. For example, it may be in the physical form of a powder, tablet, capsule, lozenge, gel, solution, suspension, syrup, or the like. In addition to the nitrogen-containing virus-inhibiting compound, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer the compound according to the method of the invention. Such pharmaceutical compositions may be administered by any known route. The term "parenteral" used herein includes subcutaneous, intravenous, intraarterial, intrathecal, and injection and infusion techniques, without limitation. By way of example, the pharmaceutical compositions may be administered orally, topically, parenterally, systemically, or by a pulmonary route.

These compositions may be administered according to the methods of the invention in a single dose or in multiple doses which are administered at different times. Because the inhibitory effect of the composition upon a virus may persist, the dosing regimen may be adjusted such that virus propagation is retarded while the host cell is minimally effected. By way of example, an animal may be administered a dose of the composition of the invention once per week, whereby virus propagation is retarded for the entire week, while host cell functions are inhibited only for a short period once per week.

The following specific examples are to be construed as merely illustrative, and not limitive, of the remainder of the disclosure.

Experimental

Synthesis of long chain N-alkyl compounds

EXAMPLE 1

Preparation of N-nonyl-DGJ (NN-DGJ), N-nonyl-methylDGJ (NN-MeDGJ), N-nonyl-altrostatin, N-nonyl-DNJ (NN-DNJ), N-nonyl-DMDP (NN-DMDP), and N-nonyl-2-aminobenzamide

The parent amino or imino compound (DGJ, MeDGJ, altrostatin, DNJ, DMDP, or 2-aminobenzamide (2ABC9) was reductively alkylated with nonylaldehyde (1.2 mol equivalents) in the presence of one mole equivalent of sodium cyanoborohydride for three hours at room temperature in acidified methanol. Typical yields from this reaction were greater than 95% as determined by amperometric detection after high performance cation-exchange chromatography (Dionex). N-Nonyl-compounds were purified from the reaction mixture by high performance liquid chromatography (HPLC) as follows. A sample was applied to a SCX cation-exchange column (7.5 x 50 mm) in 20%(v/v) acetonitrile and eluted with a linear gradient of 20% acetonitrile containing 500 mM ammonium formate, pH 4.4. The N-nonyl compound was recovered and applied to a C18 reverse-phase column (4.6 x 250 mm) equilibrated with 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA). The compound was eluted from the column using a linear gradient of 80% acetonitrile containing 0.1% trifluoroacetic acid, lyophilized to dryness, and dissolved in methanol. Samples of purified compound were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using 2,5-dihydroxybenzoic acid as the matrix.

Compounds having different N-alkyl chain lengths are prepared by replacing nonyl aldehyde with the desired chain length aldehyde. Tritiated compounds are prepared by employing tritiated sodium cyanoborohydride as the reducing agent in the reaction.

N- nonyl-DGJ

MALDI-TOF mass spectrometry showed a peak at 288.83 atomic mass units as expected for the structure shown in Figure 1.

N-nonyl-MeDGJ

MALDI-TOF mass spectrometry showed a peak at 273.9 atomic mass units as expected for the structure shown in Figure 1.

N-nonyl-altrostatin

MALDI-TOF mass spectrometry showed a peak at 289.44 atomic mass units as expected for the structure shown in Figure 1.

N-nonyl-DMDP

MALDI-TOF mass spectrometry showed a peak at 287.66 atomic mass units as expected for the structure shown in Figure 1.

N-nonyl-2-aminobenzamide (2ABC9)

MALDI-TOF mass spectrometry showed a peak at 261.57 atomic mass units as expected for the structure shown in Figure 1.

Cell toxicity

Cell toxicities in MDBK cells of various chain length N-alkyl DNJ are summarized in Table 1.

TABLE 1

N-alkyl Chain Length	% Viability at 10 μm	% Viability at 100 μm
C_4	74	77
C_5	80	70
C_6	73	71
C_8	70	71
C_9	56	41
C_{10}	73	43
C_{12}	86	1
C_{16}	88	4
C_{18}	84	2

Inhibition studies

Materials and Methods

Plaque Reduction and Yield Assays. MDBK cells were grown in six-well plates in the presence or absence of inhibitor, infected with cp BVDV (moi = 0.005; 500 pfu per well) for one hour at 37°C. The inoculum was then replaced with growth medium alone or with growth media and the antiviral agent and incubated for two or three days in the presence or absence of inhibitor (plaque reduction assay). After counting the plaques by eye under the microscope, the supernatant containing secreted

infectious virus was removed from the wells and used to infect a fresh monolayer of MDBK cells in six-well plates. After three days the resulting plaques were counted under the microscope (yield assay).

IC_{50} values for the following compounds have been determined. All the following values are approximate and given in micromolar concentrations. IC_{50} values for NN-DNJ was about 2.5 μM, NN-DGJ was about 6 μM, NN-MeDGJ was about 2.5 μM, NN-altrostatin was less than about 73 μM, Nonylamine was less than 70 μM, NN-2-aminobenzamide (ABC9) was about 18 μM, N-nonyl glucoside was greater than about 90 μM, and N-octyl glucoside was greater than about 90 μM. Some of these IC_{50} values are depicted graphically in Figure 5. The percent of BVDV plaques produced by an infected cell culture in the presence different concentrations of 2ABC9, nonylamine, NN-altrostatin, NN-DGJ, NN-MeDGJ, NN-DNJ, and NN-DMDP are shown in Figure 6.

EXAMPLE 2

Secretion of Infectious BVDV in the presence of long chain N-alkyl compounds

MDBK cells were grown to semi-confluence in individual wells of 24-well trays. The cells were then infected by BVDV by incubating the cells for one hour at 37°C in the presence of approximately 500 PFU of the NADL strain of BVDV suspended in growth medium. The inoculum was then replaced with growth medium alone or growth medium containing a particular concentration of a long chain N-alkyl compound. After three days, the supernatants were removed and used to infect fresh MDBK monolayers in six-well plates. After three days, the cell monolayers were observed microscopically before and after staining with 0.2% (w/v) crystal violet in ethanol for plaque counting, and 0.2% neutral red for viability and the presence and number of virus-induced plaques was determined. The results were expressed as percentages of the number of plaques resulting from infection with the inhibitor-free plaque assay supernatant (=100%). The results of these experiments are presented in the graphs depicted in Figure 2, Figure 3, and Figure 4. Figure 2 is a graph depicting the variation in IC_{50} for N-alkylated DNJ compounds having the following chain lengths: butyl, pentyl, hexyl, octyl, nonyl, decyl, dodecyl, hexadecyl, and octadecyl. The concentration of N-nonyl-DNJ (NN-DNJ) which caused 50% inhibition of plaque formation (IC_{50}) was determined to be approximately 7 micromolar. The value of

IC_{50} for N-decyl-DNJ was approximately 2.5 micromolar.

Inhibitory constants for various chain length N-alkyl DNJ derivatives for ceramide glucosyl transferase (CerGlcT) and α-glucoside are summarized in Table 2.

TABLE 2

N-alkyl Chain Length	CerGlcT (IC_{50}, μM)	α-Glucosidase (IC_{50}, μM)
C_4	34.4	0.57
C_5	26.8	
C_6	23.8	
C_8	16.8	
C_9	7.4	
C_{10}	3.1	0.48
C_{12}	5.2	
C_{16}	3.4	
C_{18}	4.1	

EXAMPLE 3

Uptake of radioactively labeled inhibitors by different cell types

MDBK and HepG2 cells were grown to confluency in 12-well plates and incubated in the presence of tritiated long chain N-alkylated compounds (100,000 cpm/well) for the times indicated in Figure 7. The supernatant was removed and kept. The cells were washed with PBS (2x500 μL), fixed with 500 μL of icecold 10% perchloric acid/2% phosphotungstic acid, washed twice with 500 μL of icecold ethanol, air dried, and lysed overnight at room temperature with 500 μL of 0.5 M NaOH. The percentage of radioactive counts in the supernatant, PBS wash and lysed cells was determined by liquid scintillation counting. The results are shown graphically in Figure 7.

All cited publications, books, patents, and patent applications are incorporated by reference in their entirety where they are cited.

From the foregoing, it would be apparent to persons skilled in the art that the invention can be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments should be considered only as illustrative, not restrictive, because the scope of the invention will be indicated by the appended claims rather than by the foregoing description. All modifications which come within the meaning and range of the lawful equivalency of the claims are to be embraced within their scope. In that sense, no particular order of process steps is

intended unless explicitly recited.

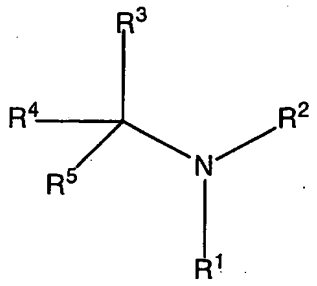
Other embodiments are within the claims.

What we claim is:

CLAIMS

1. A method of inhibiting morphogenesis of a virus comprising administering an effective amount of a nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt thereof, to a cell or an individual infected with the virus, wherein the nitrogen-containing virus-inhibiting compound includes an N-C_8-C_{16} alkyl group.
2. A method of treating an individual infected with a virus comprising administering an effective amount of a nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt thereof, to an individual infected with a virus, wherein the nitrogen-containing virus- inhibiting compound includes an N-C_8-C_{16} alkyl group.
3. The method of claim 1 or 2, wherein the nitrogen-containing virus-inhibiting compound includes an N-C_8-C_{10} alkyl group.
4. The method of claim 3, wherein the nitrogen-containing virus-inhibiting compound includes an N-nonyl group.
5. The method of claim 1 or 2, wherein the nitrogen-containing virus-inhibiting compound has an IC_{50} of about 20μM or less for the inhibition of BVDV.
6. The method of claim 1, 2 or 3 wherein the nitrogen-containing virus-inhibiting compound is an N-alkylated piperidine, an N-alkylated pyrrolidine, an N-alkylated phenylamine, an N-alkylated pyridine, an N-alkylated pyrrole, or an N-alkylated amino acid.
7. The method of claim 6 wherein the N-alkylated piperidine or the N-alkylated pyrrolidine is an imino sugar.

8. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound has the formula:



wherein:

R^1 is a C_8-C_{16} alkyl;

R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, or R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, an aroyloxy, or deleted;

R^4 is hydrogen or deleted; and

R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy, or

R^3 and R^5, together, form a phenyl and R^4 is deleted,

wherein when R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted, all Y are deleted,

or a physiologically acceptable salt or solvate of said compound.

9. The method of claim 8, wherein R^1 is a C_8-C_{10} alkyl.

10. The method of claim 9, wherein R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, R^4 is hydrogen, and R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy.

11. The method of claim 10, wherein R^3 is carboxy.

12. The method of claim 10, wherein R^3 and R^5, together, form a phenyl and R^4 is deleted.

13. The method of claim 8, wherein R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

14. The method of claim 9, wherein R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

15. The method of claim 14, wherein n is 3.

16. The method of claim 14, wherein n is 4.

17. The method of claim 14, wherein each X is hydrogen and each Y, independently, is hydroxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

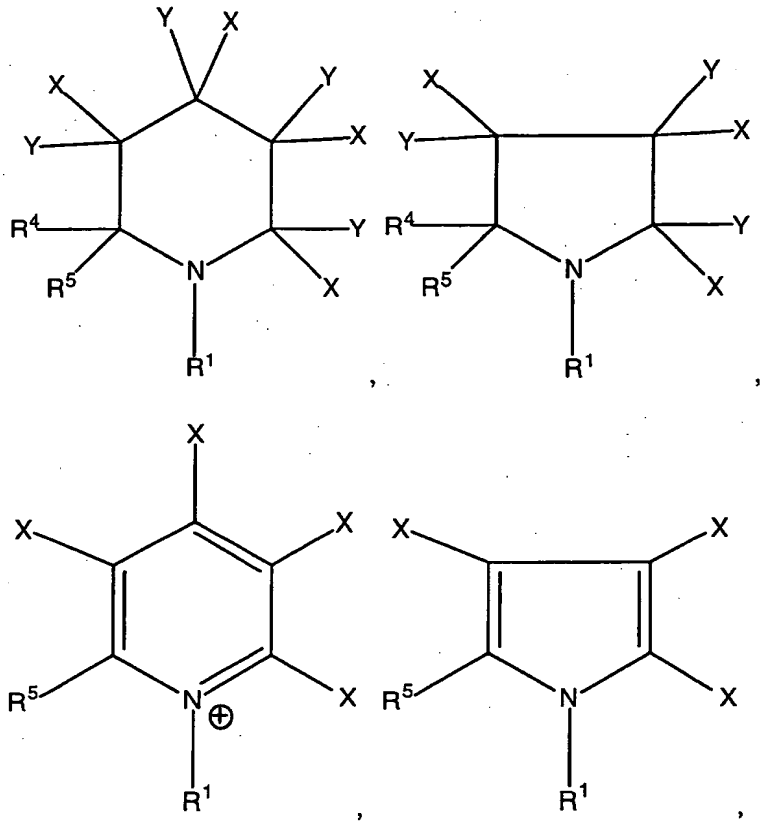
18. The method of claim 17, wherein R^4 is hydrogen and R^5 is hydrogen.

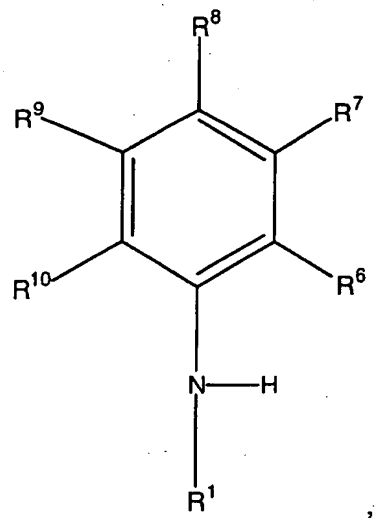
19. The method of claim 9, wherein R^4 is deleted and R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each Y is deleted, and each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4

alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

20. The method of claim 9, wherein each X, independently, is hydrogen, hydroxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

21. The method of claim 8, wherein the nitrogen-containing virus-inhibiting compound has the formula:





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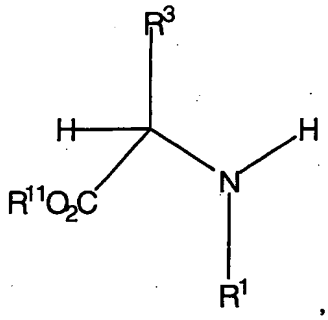


,

wherein each of R^6-R^{10}, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_4 acyloxy, or an aroyloxy, and R^{11} is hydrogen, or a C_1-C_6 alkyl.

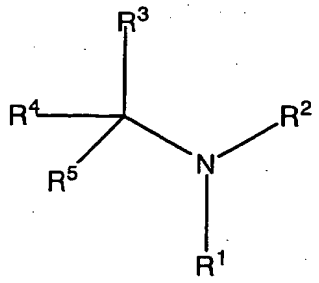
22. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound is N-nonyl DGJ, N-nonyl MeDGJ, N-nonyl altrostatin, N-nonyl DMDP, N-nonyl DNJ, or N-nonyl-2-aminobenzamide.
23. The method of claim 1, wherein the cell is a mammalian host cell.
24. The method of claim 1, wherein the cell is a human host cell.
25. The method of claim 1 or 2 wherein the individual is a mammal.
26. The method of claim 1 or 2 wherein the individual is a human.
27. The method claims 1 or 2 wherein the compound is administered orally.
28. The method of claim 1 or 2 wherein the virus is a flavivirus.
29. The method of claims 28 wherein the flavivirus is selected from the group consisting of yellow fever virus, dengue viruses 1-4, Japanese encephalitis virus, Murray Valley encephalitis, Rocio virus, West Nile fever virus, St.

Louis encephalitis virus, tick-borne encephalitis virus, Louping ill virus, Powassan virus, Omsk hemorrhagic fever virus and Kyasanur forest disease virus.

30. The method of claim 1 or 2 wherein the virus is hepatitis C virus.

31. The method of claim 1 or 2 wherein the virus is a pestivirus.

32. A compound having the formula:



wherein:

R^1 is a C_8-C_{16} alkyl;

R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, or R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, an aroyloxy, or deleted;

R^4 is hydrogen or deleted; and

R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy, or

R^3 and R^5, together, form a phenyl and R^4 is deleted,

wherein when R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted, all Y are deleted,

or a physiologically acceptable salt or solvate of said compound.

33. The compound of claim 32, wherein R^1 is a C_8-C_{10} alkyl.

34. The compound of claim 33, wherein R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, R^4 is hydrogen, and R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy.

35. The compound of claim 34, wherein R^3 is carboxy.

36. The compound of claim 34, wherein R^3 and R^5, together, form a phenyl and R^4 is deleted.

37. The compound of claim 33, wherein R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

38. The compound of claim 37, wherein n is 3.

39. The compound of claim 37, wherein n is 4.

40. The compound of claim 37, wherein each X is hydrogen and each Y, independently, is hydroxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

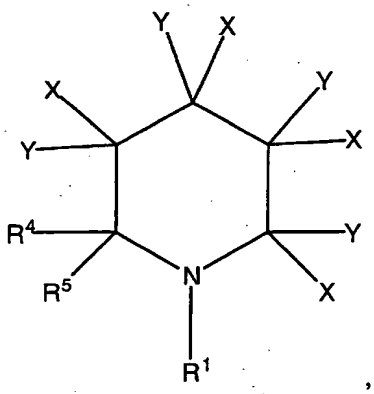
41. The compound of claim 40, wherein R^4 is hydrogen and R^5 is hydrogen.

42. The compound of claim 33, wherein R^4 is deleted and R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each Y is deleted, and each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4

alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

43. The compound of claim 33, wherein each X, independently, is hydrogen, hydroxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy.

44. The compound of claim 32, wherein the compound has the formula:



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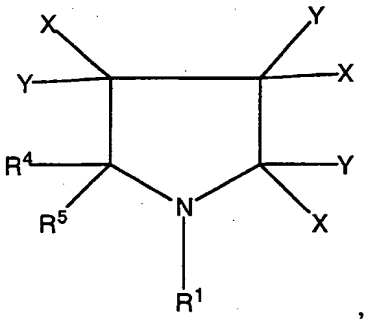

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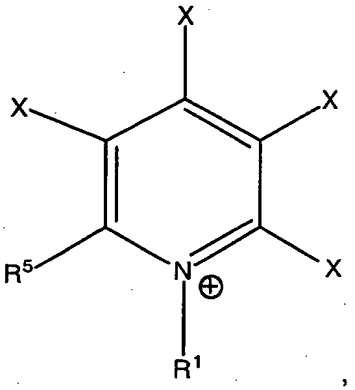

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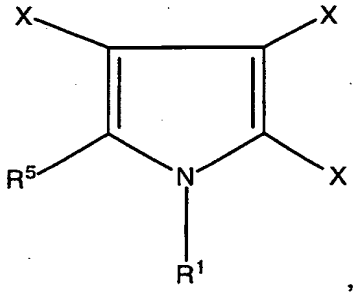

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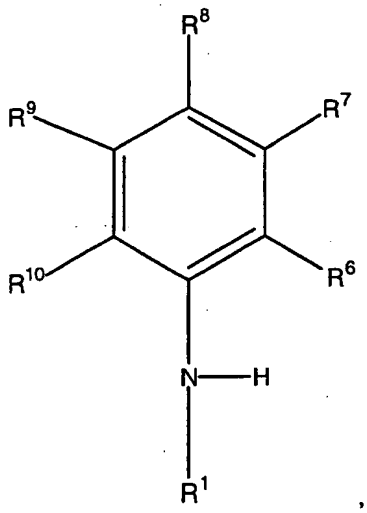

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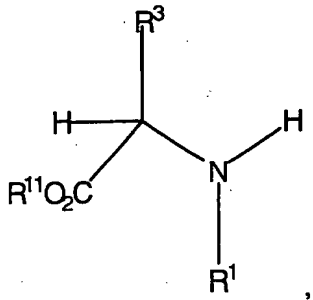

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wherein each of R^6-R^{10}, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and R^{11} is hydrogen, or a C_1-C_4 alkyl.

45. The compound of claim 32, wherein the compound is N-nonyl DGJ, N-nonyl MeDGJ, N-nonyl altrostatin, N-nonyl DMDP, N-nonyl DNJ, or N-nonyl-2-aminobenzamide.

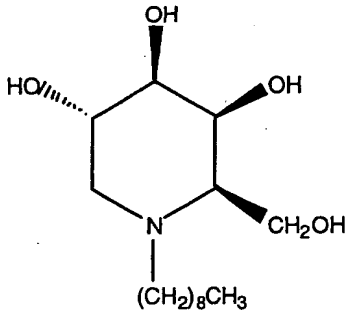
46. A pharmaceutical composition comprising a nitrogen-containing virus-inhibiting compound and a pharmaceutically acceptable carrier, wherein the nitrogen-containing virus-inhibiting compound includes an N-C_8-C_{16} alkyl group.

47. A method of manufacturing a pharmaceutical composition comprising combining a nitrogen-containing virus-inhibiting compound with a pharmaceutically acceptable carrier, wherein the nitrogen-containing virus inhibiting compound includes an N-C_8-C_{16} alkyl group.

LONG CHAIN N-ALKYL COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS THEREOF

ABSTRACT

Long chain N-alkyl amino and imino compounds and pharmaceutical compositions including long chain N-alkyl amino and imino compounds are described. The long chain N-alkyl group is a C_8-C_{16} alkyl group. The long chain N-alkyl compounds can be used in the treatment of viral infections in a cell or an individual. For example, the long chain N-alkyl compounds can be derived from a piperidine, a pyrrolidine, a phenylamine, a pyridine, a pyrrole, or an amino acid.



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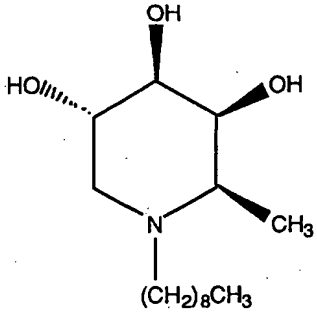
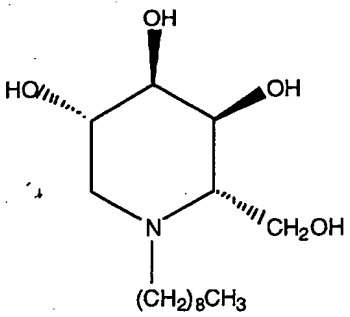
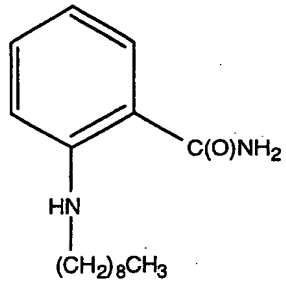


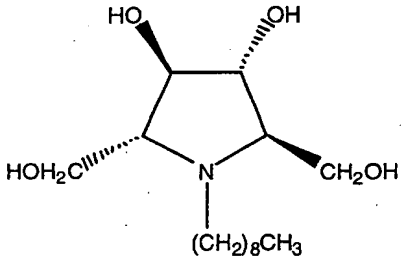
N-nonyl-MeDGJ



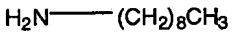
N-nonyl-altrostatin



N-nonyl-2-aminobenzamide (2ABC9)



N-nonyl-DHDP



nonylamine

FIGURE 1

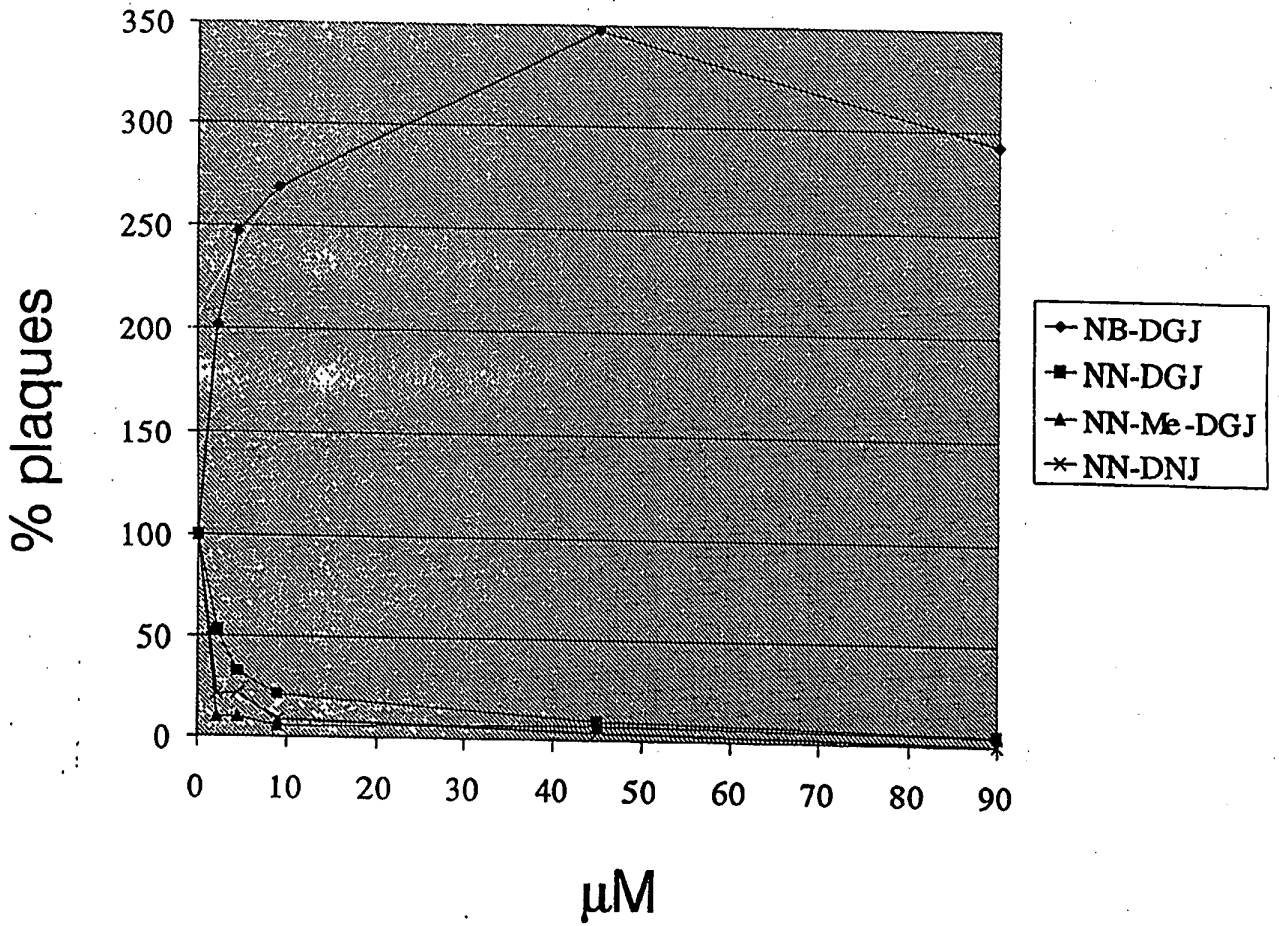


FIGURE 2

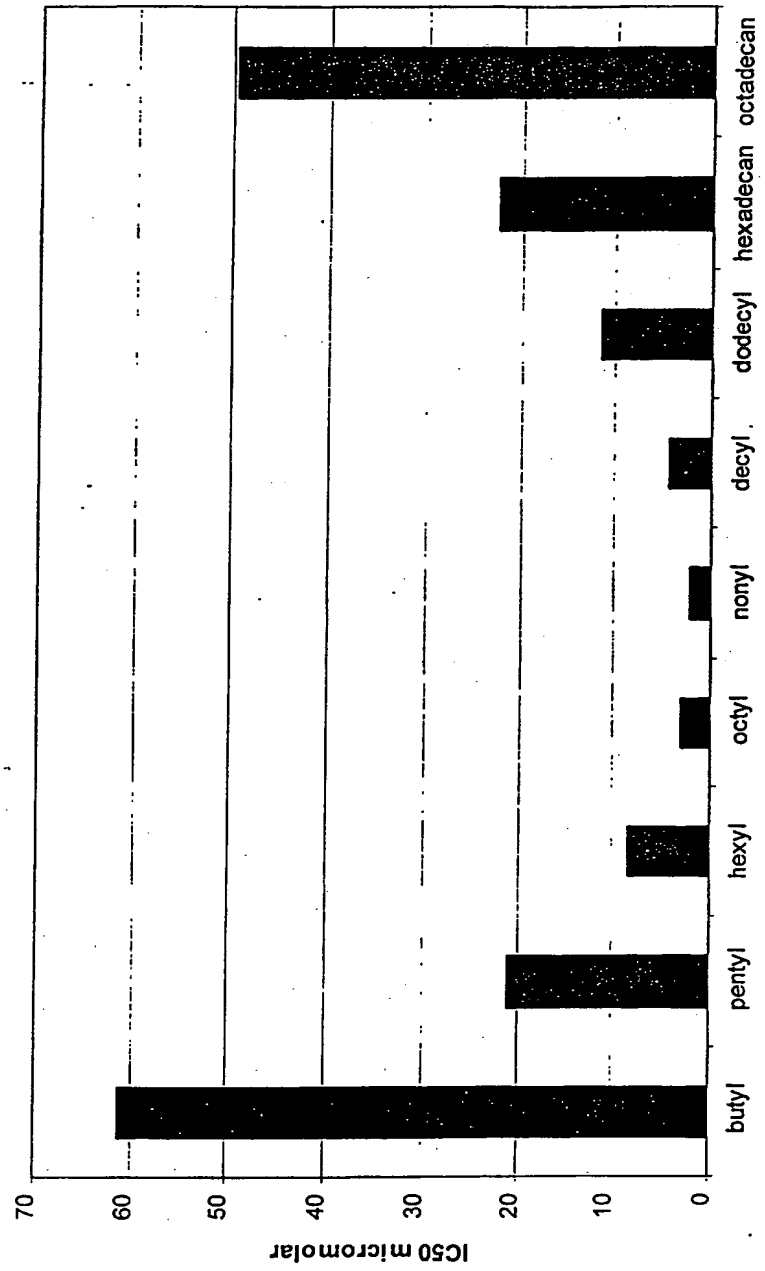


FIGURE 3

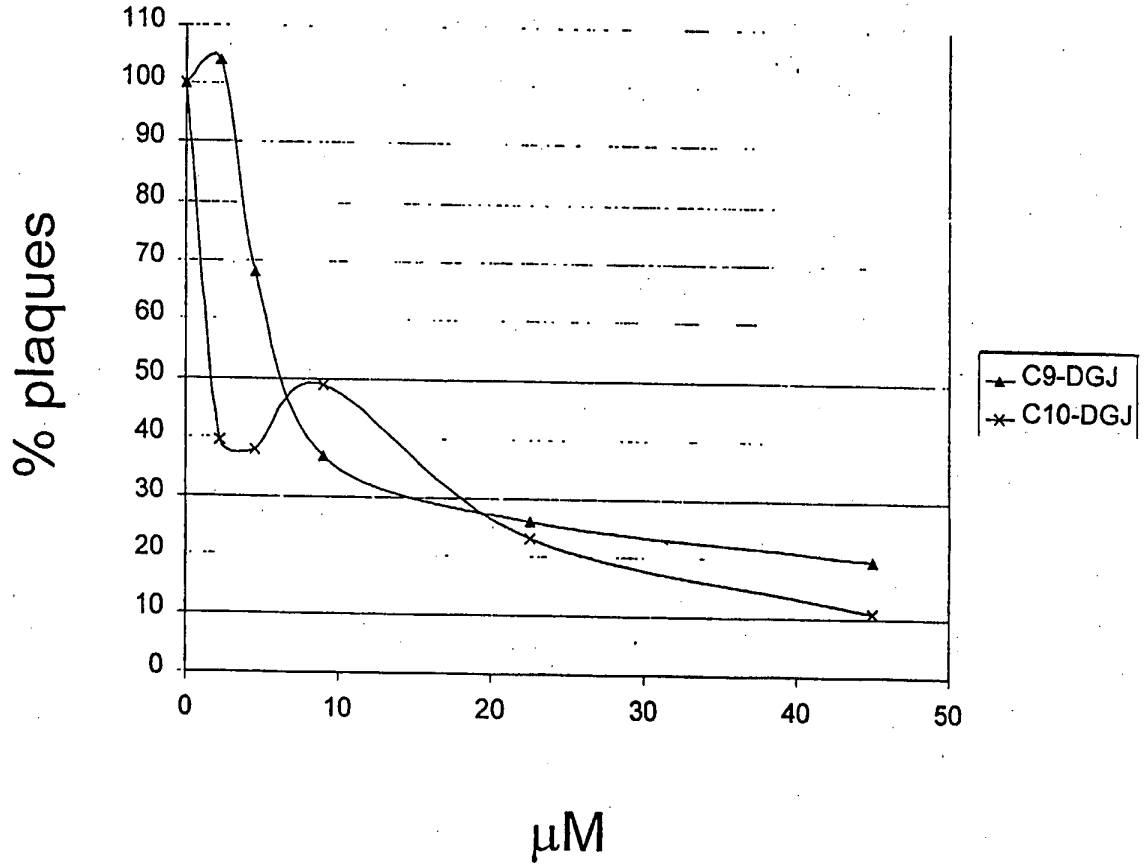


FIGURE 4

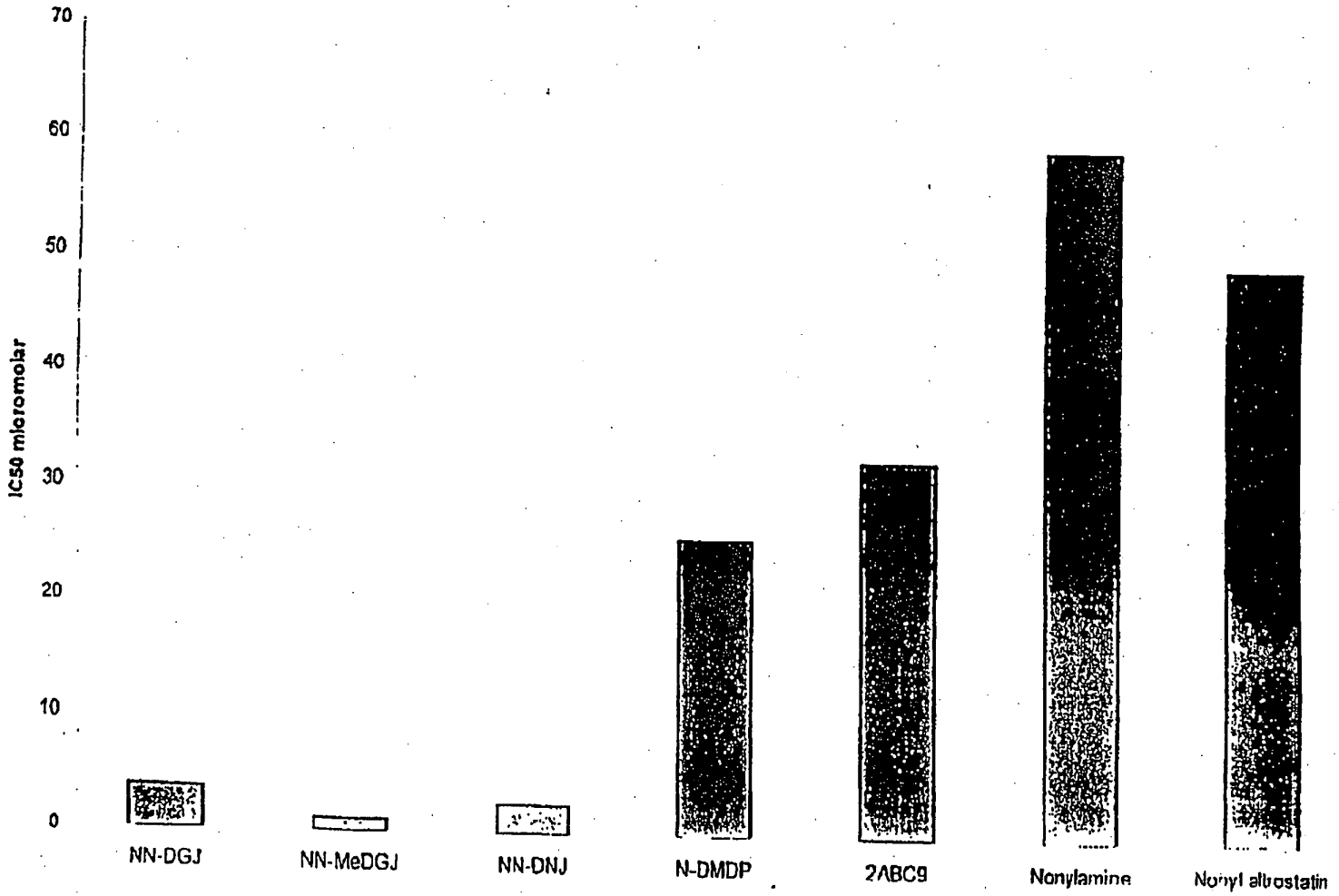


FIGURE 5

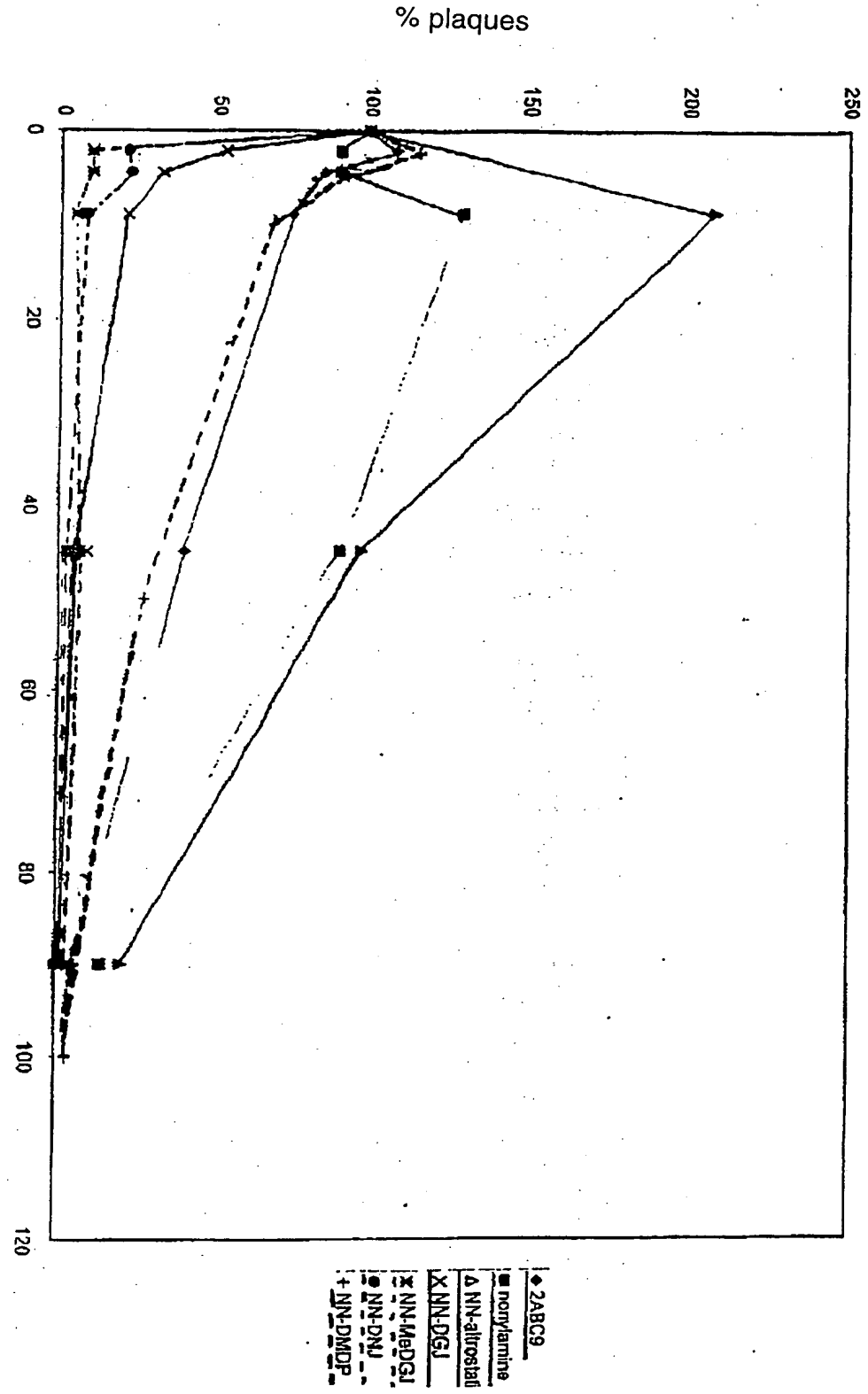


FIGURE 6

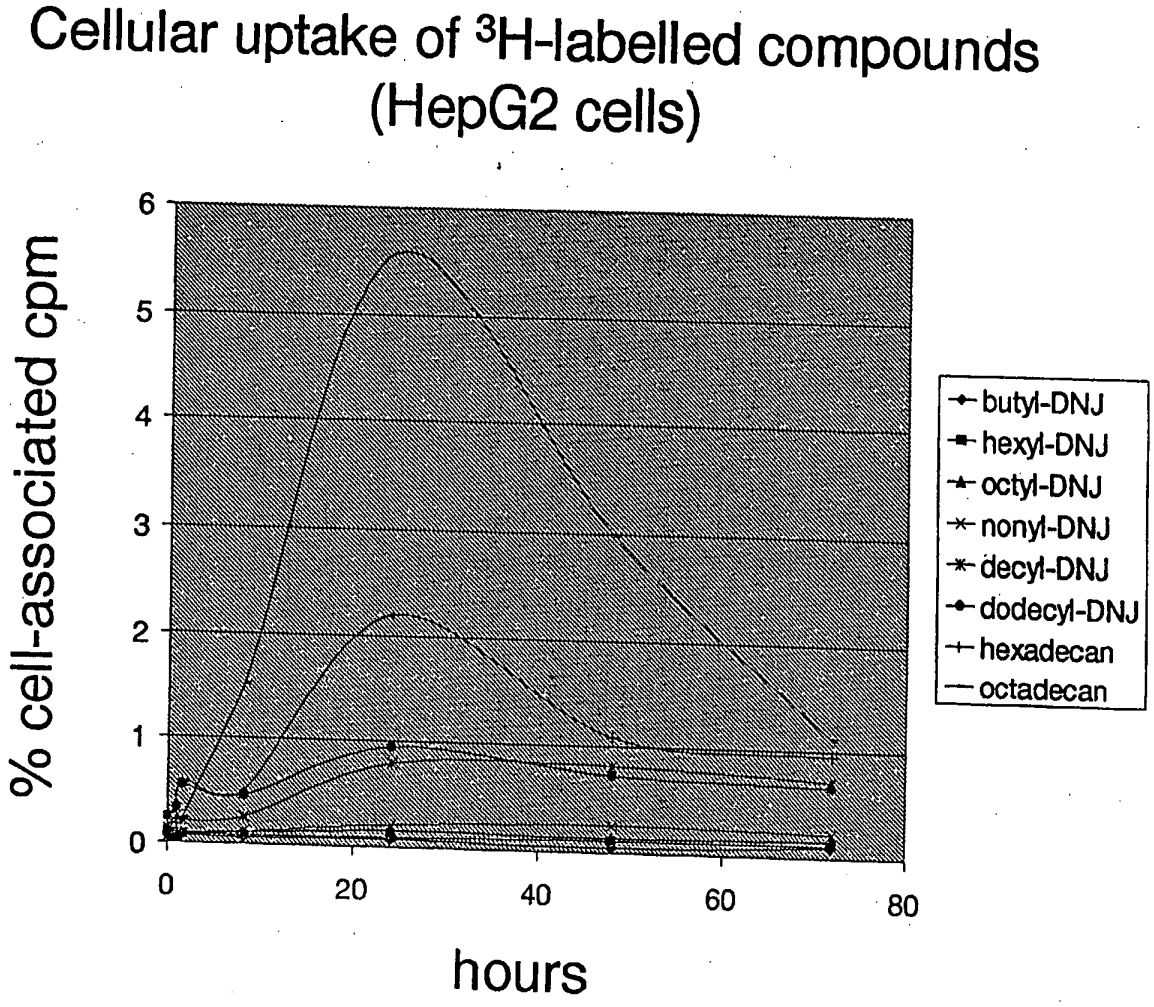


FIGURE 7